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Characterization of Cos-7 cells overexpressing the rat secretory pathway Ca^{2+}-ATPase

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Reinhardt, Timothy A., Ronald L. Horst, and W. Ray Waters. Characterization of Cos-7 cells overexpressing the rat secretory pathway Ca^{2+}-ATPase. *Am J Physiol Cell Physiol* 286: C164–C169, 2004. First published September 10, 2003; 10.1152/ajpcell.00065.2003.—On the basis of sequence similarities to the yeast PMR1 and hSPCA gene, the rat alternatively spliced mRNA has been suggested to be a Golgi secretory pathway Ca^{2+}-ATPase (SPCA). Data in this report lend further support for this hypothesis in that sucrose gradient fractionation of rat liver microsomes resulted in SPCA comigrating with the Golgi calcium binding protein CALNUC, which was well resolved from the endoplasmic reticulum marker calreticulin. Also, in PC-12 cells, antibody to SPCA colocalized with an antibody to the Golgi marker α-mannosidase II. To study the biological effects of SPCA expression, we performed stable overexpression of SPCA in COS-7 cells. Seven clones were selected for further comparison with COS-7 cells containing an empty expression vector. Overexpression of SPCA resulted in a significant reduction of plasma membrane Ca^{2+}-ATPase, sarco(endo)plasmic reticulum Ca^{2+}-ATPase, and calreticulin expression in these clones. In contrast, the expression of the Golgi calcium-binding protein CALNUC increased significantly. The phosphoenzyme intermediate formed using membranes from clone G11/5 was calcium dependent, significantly more intense than in COS-7 cells, and not affected by La^{3+} treatment. Calcium uptake by G11/5 microsomes was ATP dependent and significantly greater than in microsomes from parent COS-7 cells. The overexpression of SPCA significantly increased the growth rate of these cells compared with COS-7 cells containing only the empty vector. These data demonstrate that overexpression of the rat SPCA results in significant changes in the expression of calcium transport and storage proteins in COS-7 cells.

calcium transport

THE GOLGI COMPLEX processes newly synthesized proteins and sorts proteins destined for other cellular compartments or secretion (11). Calcium is critical for many cellular functions, including protein processing and secretion by the Golgi (3, 7). The Golgi complex contains a large calcium pool in the lactating mammary cell (5), and studies using ion microscopy or electron energy loss imaging analysis have shown that the Golgi complex is a calcium-rich cell compartment in most cells (23). The mechanism by which calcium is transported into the Golgi and maintained at high levels has not been completely defined.

In contrast, calcium transport and storage in the endoplasmic reticulum (ER) is more clearly understood. Calcium is transported into the ER by sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and maintained by a variety of ER resident calcium-binding proteins that include calreticulin and calnexin, among others (14). At least two Golgi luminal calcium-binding proteins have been identified that meet the criteria for maintaining the Golgi calcium pool, Cab45 and CALNUC (18, 19, 27). In mammalian Golgi, biochemical evidence suggests a P-type Ca^{2+}-ATPase with slightly different characteristics from the plasma membrane Ca^{2+}-ATPases (PMCA) or SERCAs (2, 7, 22, 32). More recently, the yeast PMR1 gene product was found to be a Ca^{2+}-ATPase located in the Golgi complex of yeast and *Caenorhabditis elegans* (28, 31). Rat, human, and bovine homologs of the yeast Pmr1 have been cloned (16, 17) (Reinhardt TA, unpublished data; accession no. AF-230532), and the human secretory Ca^{2+}-ATPase (SPCA) has been characterized (4, 10, 30). Mutations in the human SPCA have been shown to be the cause of Hailey-Hailey disease. Cultured keratinocytes from these patients have impaired regulation of cytoplasmic calcium (17). Taken together, the yeast and human data strongly suggest that SPCA is the mammalian Golgi Ca^{2+}-ATPase. The opposing argument put forth by Taylor et al. (29) is that the Golgi complex does not contain a unique "resident" Ca^{2+}-ATPase. They state that all calcium uptake into Golgi can be attributed to PMCAs in transit to the plasma membrane and to SERCAs that are not restricted to the ER. However, the human PMR1 homolog has recently been shown to be a Golgi Ca^{2+}-ATPase (10, 30). Studies in the rat have shown that putative rat SPCA protein is expressed in a number of tissues, with the highest expression being in the brain and lactating mammary gland (24, 25). Furthermore, these studies show that the putative rat SPCA is the only candidate Ca^{2+}-ATPase whose expression increased significantly before parturition at a time of significant calcium accumulation in the mammary gland Golgi in preparation for milk production.

In this study, we investigated the effect that overexpressing rat SPCA has on COS 7 cells. The results show that rat SPCA is located in the Golgi, forms a phosphorylated intermediate characteristic of Ca^{2+}-ATPases, and transports calcium in an ATP-dependent manner. Most significant were the findings that overexpression of rat SPCA causes significant alterations in the expression of several of the cell's calcium transport mechanisms and dramatically increases cell division.

EXPERIMENTAL PROCEDURES

Subcellular fractionation of rat liver. The National Animal Disease Center's Animal Care and Use Committee approved all animal procedures. Sprague-Dawley rats were purchased from Harlan Sprague Dawley (Madison, WI). Rats were housed individually in hanging

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basket cages on sawdust bedding. Rats were anesthetized with a 50:50 mix of CO_2-O_2 followed by decapitation. Livers were removed, weighed, placed in ice-cold 0.25 M sucrose containing protease inhibitors, and minced with scissors. Minced liver (1 g) was mixed with 5 ml of cold 0.025 M sucrose + protease inhibitors and homogenized with six to seven strokes of a Dounce homogenizer by using *pestle A*. The homogenate was then filtered through three layers of cheesecloth. This homogenate was centrifuged for 10 min at 1,800 *g* at 4°C to remove cell debris, nuclei, and mitochondria. The supernatant was diluted to its original volume and centrifuged for 60 min at 100,000 *g* at 4°C. The resulting microsomal pellet from 1 g of liver was resuspended in 1.5 ml of 55% sucrose (wt/wt) by five strokes of a Dounce homogenizer, using *pestle B*. The suspended microsomes were loaded at the bottom of a sucrose step gradient consisting of 40, 35, 30, 25, and 20% sucrose (wt/wt in 1 mM Tris·HCl, pH 7.5). The gradients were centrifuged at 85,500 *g* for 16 h using a SW28 rotor. At the end of the run, 15 fractions were collected from the bottom of the tube. These fractions were diluted with buffer and centrifuged at 100,000 *g* for 1 h. The resulting pellets were resuspended in buffer and assayed for protein. These fractions prepared by sucrose density gradient centrifugation were characterized by Western blotting using antibodies to rat SPCA, calreticulin, and CALNUC.

Immunocytochemistry. PC-12 cells were cultured on slides to 50–70% confluence. Cells were washed with PBS, fixed in ice-cold acetone (10 min), and permeabilized with 0.01% Triton X-100 (3 × 5 min). They were incubated with PBS + Superblock (Pierce Products, Rockford, IL) for 10 min and washed with PBS + 0.05% Tween 20 for 10 min. Cells were then incubated for 2 h with affinity-purified rabbit anti-rSPCA and MAb to rat α-mannosidase II. They were then washed 3 × 10 min in PBS + Tween 20 and incubated for 1 h with goat anti-rabbit FITC and goat anti-mouse Texas red conjugates. Cells were examined with a confocal microscope, and images were processed with Adobe Photoshop.

Stable transformation of COS-7 cells. The plasmid containing the putative rat SPCA was a gift from Gary Shull (University of Cincinnati, Cincinnati, OH). This plasmid was linearized with *Hin*dIII and used as a template for PCR with a sense primer 5′-ttgtagccatcaaccagagtgcagtttcggtgg-3′ and antisense primer 5′- TGCACCAGCCTGAACTAATCCCTGCTAGG-3′. The 3.5-kb PCR product was polished, and the blunt-ended PCR product was cloned into the pCMV-Script vector from Stratogene (La Jolla, CA). This plasmid was transformed into Epicircuran XL10 Gold Ultracompetent cells (Stratogene). Positive clones were checked for orientation and then sequenced to confirm that no changes resulted from the PCR step. The plasmid containing the rat SPCA and the empty vector pCMV were each transfected into 50% confluent COS-7 cells using GenePORTER (Gene Therapy Systems, San Diego, CA) according to the manufacturer's instructions. Cells from both transfections were placed under G418 selection (600 μg/ml) and then cloned by limiting dilution. Expanded clones were checked for overexpression of the putative rat SPCA by Western blotting by using an anti-rat SPCA (24) and comparing them to COS-7 cells containing the empty vector pCMV.

Microsomal membrane preparation. Cell microsomes were prepared as previously described (6). Briefly, cells were homogenized in 10 volumes of *buffer A*, which contained 10 mM Tris·HCL, 2 mM $MgCl_2$, 0.1 mM PMSF, 1 mM EDTA, 4 μg/ml aprotinin, and 4 μg/ml leupeptin at pH 7.5. The homogenate was mixed with an equal volume of *buffer B* (*buffer A* plus 0.3 M KCl) and centrifuged at 4,000 *g* for 10 min. The supernatant was collected, adjusted to 0.7 M KCl by the addition of solid KCl, and centrifuged at 100,000 *g* for 1 h. The supernatant was discarded, and the pellets were resuspended in *buffer C* (*buffer A* plus 0.15 M KCl). Membrane preparations were stored at −70°C until assayed. Proteins were determined using the Bio-Rad protein assay kit with a BSA standard.

Gel electrophoresis and Western blotting. The methods were basically as described previously (12). Briefly, microsomes were incubated for 15 min at room temperature in a modified Laemmli buffer containing 150 mg/ml urea and 65 mM 1,4-dithiothreitol (DTT). Samples were then electrophoresed for 1.5 h at 125 volts in a 6% Tris-glycine gel (Invitrogen, Carlsbad, CA). Proteins were transferred to nitrocellulose membranes for 1 h at 25 V in 0.7 M glycine and 0.025 M Tris at pH 7.4. Blots were developed with Pierce's Super-Signal (Pierce Products, Rockford, IL) using the protocol provided by the manufacturer. Anti-PMCA antibody 5F10 was a gift from John Penniston (Mayo Foundation, Rochester, MN). CALNUC antibody was a gift from M. Farquhar (University of California, La Jolla, CA). Additional antibodies were purchased from Affinity Bioreagents (Golden, CO). The rat SPCA antibody was prepared and characterized as described in Ref. 24, where it was shown that excess peptide eliminates specific binding in Western blots. Anti-rat SPCA used in the immunocytochemistry described above was affinity purified as follows. The cysteine containing SPCA peptide VARFQKIPNVENETIMIC used to prepare the antibody to SPCA was conjugated to a SulfoLink column from Pierce Biotechnology. (Rockford, IL) according to the manufacturer's instructions. Before affinity chromatography, 1 ml of serum was treated with 0.82 ml of saturated ammonium sulfate and 2 mg of EDTA. The sample was mixed, incubated at room temperature for 15 min, and centrifuged at 14,000 *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in 1 ml of PBS containing 0.1% NP-40, which was applied directly to the SulfoLink column containing the SPCA peptide. Chromatography was performed according to the manufacturers instructions, and this affinity-purified antibody was used in the immunocytochemistry studies.

Formation of the phosphoenzyme intermediate. Membrane proteins from either pCMV or pSPCA cells were resuspended in 20 mM MOPS-KOH, pH 6.8, and 100 mM KCl in the presence or absence of 100 μM Ca^{2+} and/or 100 μM La^{3+}. The reaction was started with 0.3 μM [γ^{32}P]ATP on ice and stopped 30 s later by the addition of ice-cold 6% trichloroacetic acid containing 1 mM ATP and 10 mM inorganic phosphate. The precipitates were washed three times with the stop solution. The proteins in the washed pellet were dissolved in electrophoresis buffer and separated on acidic gels (1, 26). The gel was dried and imaged on an Instantimager (Packard Instruments, Downers Grove, IL).

^{45}Calcium uptake. The influx of ^{45}Ca into microsomes prepared from cells containing empty vector (pCMV cells) and cells overexpressing rat SPCA was measured using a modification of the fast Millipore filtration method (8). Briefly, 20 ug of microsomal membrane proteins were resuspended in transport buffer (25 mM TES-TEA, pH 7.2, 160 mM KCL, 40 mM KH_2PO_4, 7 mM $MgCl_2$, 5 mM NaN_3, 100 uM $CaCl_2$, 4 ug/ml oligomycin, and 500 uM ouabain). The free Ca^{2+} concentration was adjusted with EGTA (0–0.5 mM) using the program described (9). The ^{45}Ca uptake assay was linear for 12–15 min, so a 5-min assay period was used (data not shown). Microsomes plus ^{45}Ca (3×10^6 cpm) were preincubated at 37°C for 3 min. The reaction was started with the addition of 6 mM ATP (final concentration), with some tubes receiving no ATP and serving as controls. Five minutes after the addition of ATP, the mixture was rapidly filtered through a 0.45-μm nitrocellulose filter and washed four times with wash buffer (10 mM Tris·HCl, 150 mM KCl, and 1 mM $CaCl_2$). The filters were then counted in a Beckman liquid scintillation counter.

Cell growth. Control cells and cells overexpressing rat SPCA were plated at 500,000 cells/well at *time zero*. Cells were harvested at the indicated times, washed, and counted using a Coulter counter.

RESULTS

Rat SPCA localizes to the Golgi in rat liver and PC-12 cells. Rat liver microsomes were fractionated on a sucrose density gradient. Western blotting of these fractions demonstrated that

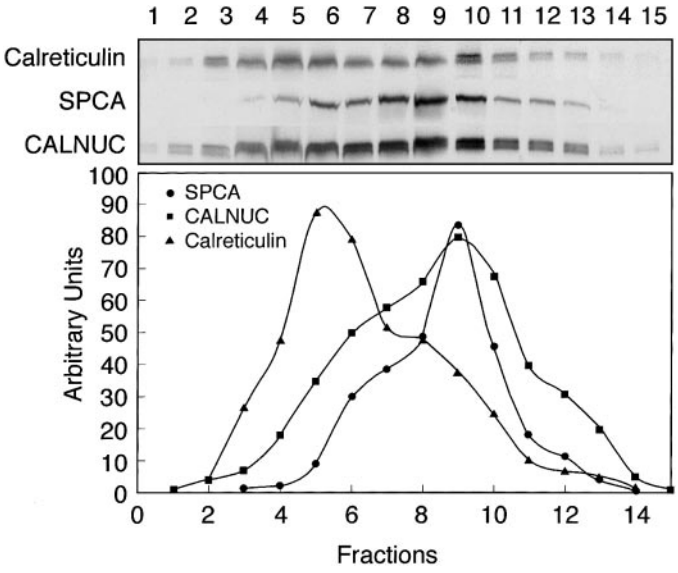

Fig. 1. Subcellular fractionation of rat liver microsomes in sucrose density gradients. Centrifugation of rat liver microsomes for 16 h at 85,000 *g* in sucrose gradients was followed by collection of 15 fractions from the bottom of the tube. These fractions were subjected to SDS-PAGE and Western blotting. Secretory pathway Ca^{2+}-ATPase (SPCA) cosedimented with the Golgi protein CALNUC and was well resolved from the endoplasmic reticulum (ER) marker calreticulin.

the rat SPCA cosediments with the Golgi localized calcium-binding protein CALNUC (Fig. 1). CALNUC and rat SPCA were well resolved from the ER marker protein calreticulin in these sucrose gradient fractions. PC-12 cells, which have a well-defined Golgi, were examined by confocal microscopy to further investigate the subcellular location of rat SPCA. It can be seen that the focus of cellular fluorescence in PC-12 cells is the same for rat SPCA and the Golgi marker α-mannosidase II (Fig. 2)

Stable overexpression of the rat SPCA in COS-7 cells. Primary selection with G418 (600 ug/ml) yielded numerous primary clones. After a second round of selection and limited dilution cloning, the expanded clones were examined by Western blotting for rat SPCA expression and compared with stably transformed COS-7 cells containing the empty cloning vector pCMV. Figure 3 shows the results for seven rat SPCA-positive clones. All clones expressed significantly more rat SPCA than

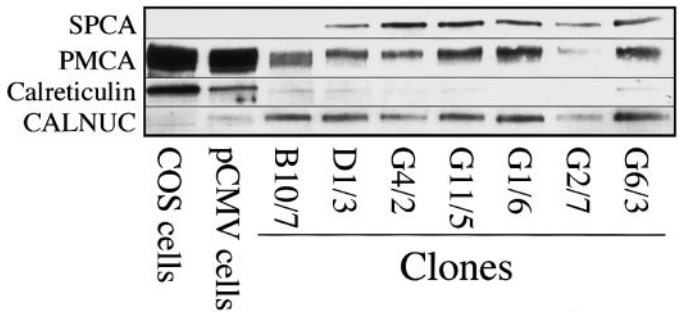

Fig. 3. Western blots of microsomes prepared from COS 7, pCMV (empty vector) cells, and 7 clones overexpressing SPCA. The first panel shows the expression of SPCA in the 7 clones compared with the parent cell line. The second, third, and fourth panels show similar results for PMCA, calreticulin, and CALNUC, respectively. The amount of membrane protein loaded was 30 μg/lane.

the control pCMV cells, which express little SPCA, as do normal COS-7 cells (data not shown). The overexpression of a putative Ca^{2+} transporter in cells might be expected to result in significant changes in the expression of other calcium transporters and binding proteins. Figure 3*A* shows that the plasma membrane calcium transporter PMCA and the ER calcium-binding protein calreticulin are dramatically downregulated in clones overexpressing SPCA. Film scanning showed a range of downregulation for PMCA in the clones of 6–18 times, with PMCA in clone G2/7 nearly undetectable with these assay conditions. The range for calreticulin downregulation in the clones was 7–32 times and undetectable in clones G2/7 and G1/6. In contrast, the expression of the Golgi calcium-binding protein CALNUC is markedly increased two to six times in the SPCA clones. From these findings, clone G11/5 was selected for further study due to its high level of SPCA expression. The other clones were not examined further.

Formation of a phosphorylated intermediate by rat SPCA. The formation of the phosphorylated intermediate of the reaction cycle was studied on microsomes from stably transformed COS-7 cells containing the empty cloning vector pCMV and clone G11/5 overexpressing rat SPCA. The phosphorylated intermediate formed by cells overexpressing rat SPCA was calcium dependent and more intense than for pCMV cells (Fig. 4). The addition of La^{3+} to the assay buffer inhibited the SERCA-phosphorylated intermediate in pCMV cells and re-

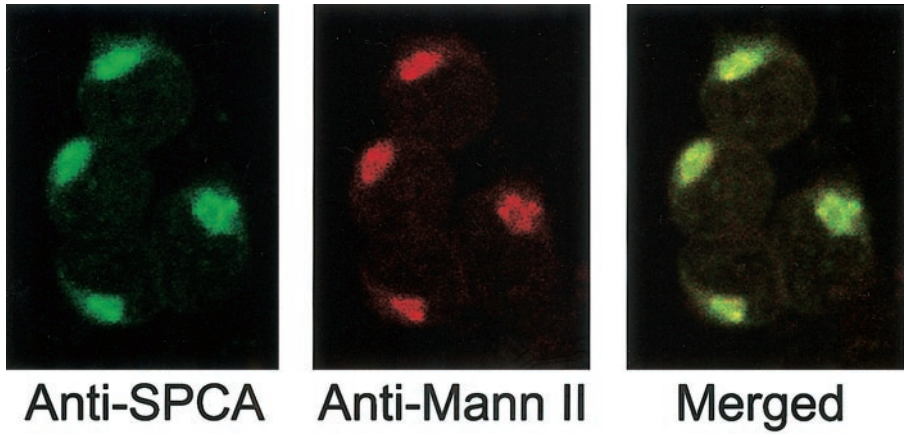

Fig. 2. Fluorescence microscopy of PC-12 cells. Fixed and permeabilized PC-12 cells were incubated with rabbit anti-SPCA, followed by goat anti-rabbit FITC conjugate, and with mouse anti-mannosidase II, followed by goat anti-mouse Texas red conjugate. These cells were then examined by confocal microscopy. *Left:* green fluorescence for SPCA in the cells. *Middle:* red fluorescence for mannosidase II in the cells. The focus of cellular fluorescence is the same for rat SPCA and the Golgi marker mannosidase II. This is confirmed at *right,* where the green and red images are merged to yield yellow where both proteins coreside.

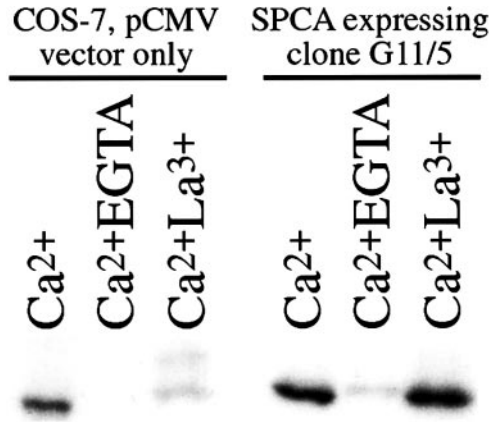

Fig. 4. Formation of the phosphoenzyme intermediate for Ca^{2+}-ATPases in microsomes from parent cells compared with microsomes from cells overexpressing SPCA (clone G11/5). *Left*: results for the parent cell microsomal proteins phosphorylated in the presence or absence of free Ca^{2+}, as well as in the presence of both Ca^{2+} and La^{3+}. *Right*: results for the clone G11/5 cell microsomal proteins phosphorylated in the presence or absence of free Ca^{2+}, as well as in the presence of both Ca^{2+} and La^{3+}. The blot shown is representative of 3 experiments.

sulted in the appearance of a faint PMCA-phosphorylated intermediate in these cells. In cells overexpressing SPCA, the addition of La^{3+} did not affect the intensity of the phosphorylated intermediate, and no faint PMCA phosphorylated intermediate was observed. Because SERCA and SPCA are similar in size, these results suggest that SERCA is downregulated in cells overexpressing rat SPCA and that the formation of the SPCA-phosphorylated intermediate is resistant to La^{3+}. Figure 5 confirms by Western blotting that SERCA is dramatically downregulated in clone G11/5, as well as shows that the expression of PMCA, CALNUC, and calreticulin remain the same after numerous cell passages compared with early passage cells shown in Fig. 3.

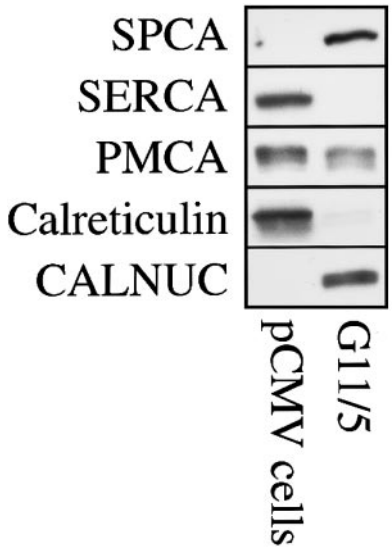

Fig. 5. Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), plasma membrane Ca^{2+}-ATPase (PMCA), CALNUC, and calreticulin expression in clone G11/5 after ~50 passages of this clone. The amount of membrane protein loaded was 30 μg/lane. The blot shown is representative of 3 experiments.

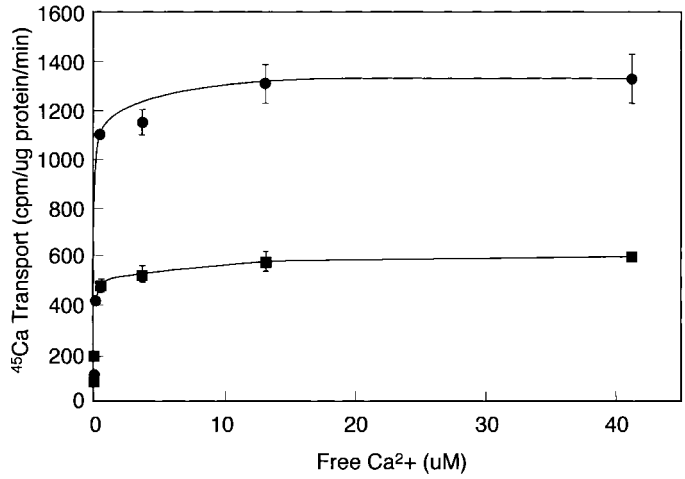

Fig. 6. Ca^{2+}-dependent uptake of Ca^{2+} by microsomes prepared from parent (empty vector) cells and cells overexpressing SPCA. Microsomes obtained from parent cells (■) and overexpressing cells (●) were preincubated with increasing amounts of free Ca^{2+} for 3 min at 37°C in the presence of ^{45}Ca (3×10^6 cpm). Transport was started by the addition of 6 mM ATP and measured for 5 min. Parallel tubes received no ATP, and non-ATP-dependent Ca^{2+} uptake was subtracted from the results before plotting. Each point is the mean of 4 measurements.

Calcium-dependent Ca^{2+} uptake by microsomes from vector only and cells overexpressing rat SPCA. Figure 6 shows the uptake of ^{45}Ca in vector-only cells compared with cells overexpressing rat SPCA. The rate of ^{45}Ca uptake by SPCA cells was twice that observed in vector-only cells. Thapsigargin treatment of microsomes from vector-only cells reduced calcium uptake by 85% compared with only 50% in cells overexpressing SPCA (Fig. 7). ^{45}Ca uptake by microsomes from both control and SPCA overexpressing cells was equally sensitive to vanadate and completely inhibited by 40 uM vanadate (data not shown).

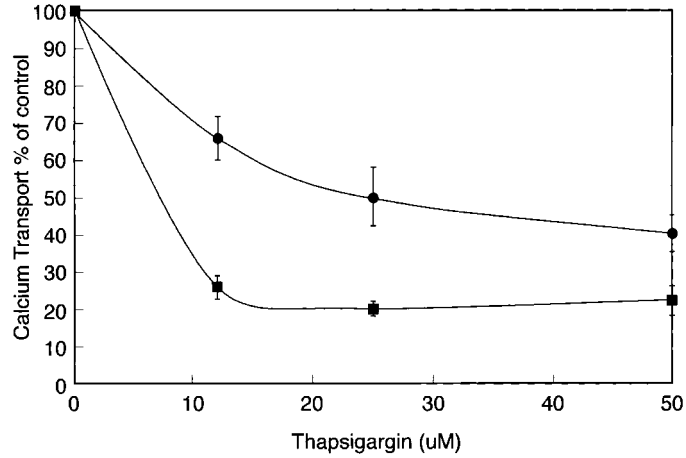

Fig. 7. Effect of thapsigargin on uptake of Ca^{2+} by microsomes prepared from parent (empty vector) cells and cells overexpressing SPCA. Microsomes obtained from parent cells (■) and overexpressing cells (●) were preincubated for 3 min at 37 C in the presence of ^{45}Ca (3×10^6 cpm) and increasing amounts of thapsigargin. Transport was started by the addition of 6 mM ATP and measured for 5 min. Parallel tubes received no ATP, and non-ATP-dependent Ca^{2+} uptake was subtracted from the results before plotting. Each point is the mean of 4 measurements.

Growth of COS-7 cells containing only vector compared with cells overexpressing SPCA. During the early phases of this work, it was noticed that the cells overexpressing SPCA were growing much faster than the control cells. It can be seen in Fig. 8 that the cells overexpression SPCA grew more rapidly that the control cells.

DISCUSSION

The Golgi is a calcium-rich compartment in the cells with Golgi-specific calcium binding proteins. Previous work has shown that the putative rat SPCA mRNA and protein are highly expressed in the lactating mammary gland (24, 25). Furthermore, the pattern of rat SPCA expression is consistent with the biochemical evidence for a Golgi Ca^{2+}-ATPase (2, 3, 7, 21, 22) in mammary tissue. In this study, we demonstrate that the rat SPCA in located in the Golgi with characteristics of a Ca^{2+}-ATPase. Rat SPCA is expressed at high levels in lactating mammary tissue (24), and our data show that high expression of SPCA in COS 7 cells results in significant adaptive changes in the expression of several cell calcium transporters and calcium binding proteins, as well as increased cell growth.

The rat SPCA is located in the Golgi and mediates high-affinity, ATP-dependent, thapsigargin-resistant calcium transport (Figs. 1–8). Calcium transport is dependent on free calcium. These findings show that rat SPCA has characteristics similar, if not identical, to that of both the Golgi Ca^{2+}-ATPase PMR1 and the recently characterized human SPCA (4, 10, 28, 30, 31).

A striking finding in the present study was the extensive adaptation of other Ca^{2+}-ATPases and calcium-binding protein expression in cells overexpressing the rat SPCA. Total PMCA protein expression was reduced 6- to 18-fold along with a 7- to 32-fold reduction in the expression of the ER calcium-binding protein calreticulin (Fig. 3). These changes, without obvious adverse effects on the cells, suggest a redundant flexibility in the cells' calcium homeostatic mechanism. The pumping of excess cytoplasmic calcium out of the cell or into the ER was necessarily reduced to compensate for increased calcium movement into the Golgi. The Golgi of these cells compensated for this increased calcium influx by increased expression of the Golgi calcium-binding protein CALNUC (Fig. 3). This would seem to be a necessary adaptation to prevent calcium cytotoxicity. Similar adaptive changes in calcium transporting pathways have been observed in cells overexpressing PMCA1a. In these cells, the activity of the CRAC pathway was upregulated, whereas IP_3R and the SERCA pumps were downregulated (20). This downregulation of SERCA is first suggested by the reduced expression of calreticulin in cells overexpressing SPCA and further supported by the phosphorylated intermediate data in Fig. 4. La^{3+} partially inhibits the formation of the SERCA phosphorylated intermediate which generally allows the PMCA-phosphorylated intermediate to become visible in this assay (14). This SERCA phosphorylation intermediate inhibition was seen in COS-7 cells containing only the empty vector pCMV. In this assay, SERCA and SPCA comigrate on the gel. The La^{3+} treatment had no effect on the phosphorylated intermediate in cells overexpressing rat SPCA, indicating that SERCA has been downregulated. The downregulation of SERCA in cells overexpressing SPCA was confirmed by Western blotting (Fig. 5). Furthermore, the phosphorylated intermediate of SPCA is resistant to the effects of La^{3+}, as has been shown for PMCA (14).

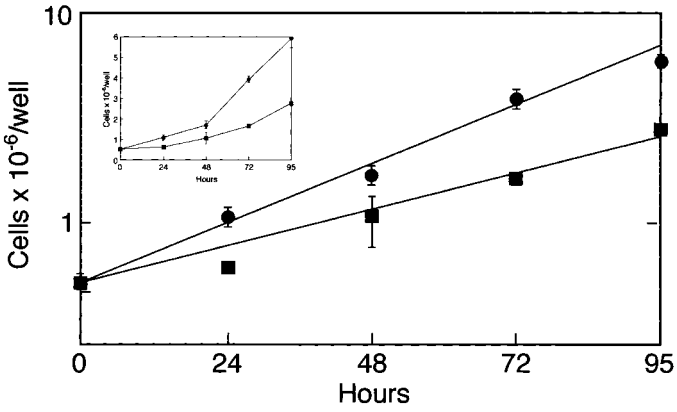

Fig. 8. Comparison of cell growth by parent (empty vector) cells and cells overexpressing SPCA (clone G 11/5). Parent cells are represented by (■) and SPCA overexpressing cells by (●) in this semilogarithmic plot of cell growth. The *inset* is a linear plot of the same data. The values reported are the mean of 4 independent measurements.

Overexpression of rat SPCA in COS-7 cells resulted in dramatic shifts in calcium transport pathways without adverse effects on cell health. These cells have been passaged more than 50 times without any change in their characteristics, including a significant increase in their rate of cell division over the parent COS-7 cells containing the empty expression vector pCMV (Fig. 8) or COS-7 cells (data not shown). Stable overexpression of PMCA in Chinese hamster ovarian (CHO) cells delayed their recovery from trypsinization, but they grew at the same rate as their parent cells after this initial delay (15). When PMCA1a was stably overexpressed in rat aortic endothelial cells, no effects on cell growth were mentioned by the authors (20). Because of the global effects of cell calcium on cellular processes, it is difficult to speculate why the overexpression of SPCA has such a dramatic effect on cell division in stably transformed COS-7 cells. Intracellular calcium pools have been shown to be essential to cell cycle function and cell growth (13). Furthermore, cells adapted to growth in thapsigargin express large amounts of thapsigargin-resistant Ca^{2+}-ATPase (33). SPCA could be the described thapsigargin-resistant Ca^{2+}-ATPase in these cells. Further studies with these interesting cells may yield clues to uncontrolled cell growth or SPCA role in cell growth.

In summary, the present study has shown that the putative rat SPCA is a Golgi Ca^{2+}-ATPase. These findings further support the significance of this protein in mammary gland function because SPCA is the only Ca^{2+}-ATPase in the mammary that upregulates just before milk production when the Golgi is storing calcium in preparation for milk production. The overexpression of SPCA highlights the flexibility and adaptability of the cells calcium transport and binding proteins to maintain calcium homeostasis and raises questions about the role of this protein in control of cell division.

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